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(54) Title: TRANSCRIPTION FACTOR-DNA BINDING ASSAY

(57) Abstract

Pharmacological agents active at the level of gene transcription are identified in high throughput drug screening assays. The methods involve combining a labeled transcription factor, a nucleic acid coupled to a ligand, a candidate pharmacological agent and a receptor immobilized on a solid substrate, such as a microtiter plate, filter, or bead. The nucleic acid has at least that portion of a nucleotide sequence naturally involved in the regulation of the transcription of the gene which is necessary for sequence-specific interaction with the transcription factor. The resultant combination is incubated under conditions whereby the receptor is bound to the ligand and, but for the presence of said candidate pharmacological agent, the transcription factor is sequence-specifically bound to the nucleic acid. Unbound transcription factor is then removed or washed from the solid substrate and labelled, sequence-specifically bound transcription factor is detected. Incubates which include candidate agents which alter transcription factor binding deviate from control incubates in terms of label signal - typically, binding is disrupted and the signal is diminished. In a preferred embodiment, the entire process is performed by a computer-controllable electromechanical robot with an axial rotatable arm.

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Transcription Factor-DNA Binding Assay

INTRODUCTION

Field of the Invention

The field of this invention is assays for screening for drugs which interfere with sequence-specific protein-DNA binding.

Background

In most populations 90-95% of adults reveal evidence of Epstein Barr virus (EBV) infection. Infection generally occurs early in life (age < 6 years) and is largely asymptomatic. Infection of college bound adults (approx. 1% per year) results in infectious mononucleosis (IM) in 30-45% of the infected individuals. IM is usually a self-limiting lymphoproliferative disease characterized by fever, malaise, and fatigue, which is rarely fatal. Effective therapeutics are entirely unavailable for EBV disease.

Gene-specific transcription factors provide a promising class of targets for novel therapeutics directed to EBV diseases for the following reasons. Transcription factors offer substantial specificity: each and every factor offers unique molecular surfaces to target.

Identifying and developing new pharmaceuticals is a multibillion dollar industry in the U.S. alone. Urgently needed are efficient methods of identifying pharmacological agents or drugs which are active at the level of gene transcription. If amenable to automated, cost-effective, high throughput drug screening, such methods would have immediate application in a broad range of domestic and international pharmaceutical and biotechnology drug development programs.

Relevant Literature

Kemp et al. (1989) Amplified DNA Assay. PCT International Application Number 46637/89 (Filed 8 December 1989).

Kemp et al. (1990) "Simplified colorimetric analysis of polymerase chain reactions: detection of HIV sequences in AIDS patients", *Gene* **94**:223-228.

Kemp et al. (1989) "Colorimetric detection of specific DNA segments amplified by polymerase chain reactions", *PNAS USA* **86**:2423-2427.

SUMMARY OF THE INVENTION

The invention provides methods for screening for pharmacological agents active at the level of gene transcription.

In general, the methods involve combining a labeled protein, a nucleic acid, a candidate pharmacological agent and a receptor immobilized on a solid substrate, such as a microtiter plate. The labelled protein includes at least a portion of a natural transcription factor involved in the regulation of the gene's expression. The nucleic acid has at least that portion of a nucleotide sequence naturally involved in the regulation of the transcription of the gene which is necessary for sequence-specific interaction, direct or indirect, with the transcription factor. The nucleic acid is conjugated to a ligand capable of specifically binding the immobilized receptor. The resultant mixture is incubated under conditions whereby the receptor is bound to the ligand and, but for the presence of said candidate pharmacological agent, the transcription factor is sequence-specifically bound to the nucleic acid. Unbound transcription factor is then removed or washed from the solid substrate and labelled, sequence-specifically bound transcription factor is detected. Binding reactions, "incubates", which include candidate agents which alter transcription factor binding deviate from control incubates in terms of label retained on the substrate - typically, binding is disrupted and the signal is diminished. In this way, pharmacological agents which modulate transcription factor-gene interactions are identified.

A wide variety of alternative embodiments of the general method are disclosed. These include a variety of labels, ligands, receptors, genes, transcription factors, auxiliary factors, etc. In a preferred embodiment, the transcription factor is viral or eukaryotic, the label is a radioactive atom, the

receptor is avidin and the ligand is biotin. Much of the method is amenable to performance by electromechanical robot. In a preferred embodiment, the method is performed by a computer-controllable electromechanical robot with an axial rotatable arm. In addition, the invention provides kits for drug screening based on the disclosed nucleic acid binding methods.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1: Schematic of robotic station design.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides efficient methods of identifying pharmacological agents or drugs which are active at the level of gene transcription. The methods are amenable to automated, cost-effective high throughput drug screening and have immediate application in a broad range of domestic and international pharmaceutical and biotechnology drug development programs.

Target diseases are limited only in that disease or disease progression be subject to inhibition by alteration of the specific interaction of a transcription factor and a gene or gene regulatory region. As such, target diseases include viral, bacterial and fungal infections, metabolic disease, genetic disease, cell growth and regulatory disfunction, such as neoplasia, inflammation, hypersensitivity, etc. The target diseases may be afflictions of plants, especially agricultural crops, or animals, especially livestock, house animals and humans.

Transcription factors are capable of sequence-specific interaction with a portion of a gene or gene regulatory region. The interaction may be direct sequence-specific binding where the transcription factor directly contacts the nucleic acid or indirect sequence-specific binding mediated or facilitated by other auxiliary proteins where the transcription factor is tethered to the nucleic acid by a direct nucleic acid binding protein. In addition, some transcription factor demonstrate induced or synergistic binding. A broad range of transcription factor-nucleic acid complexes provide useful targets. The gene and/or transcription factor may be derived from a host or from an infectious or parasitic organism. As examples, a host may be immunomodulated (e.g. by controlling inflammation or hypersensitivity) by modulating the DNA binding of a transcription factor involved

in immune cell activation; or viral, bacterial, or other microbial disease progression may be inhibited by disrupting the DNA binding of a host, viral or other microbial transcription factor involved in viral or other microbial gene transcription.

Applicable host and viral or microbial transcription factors and corresponding oligonucleotide targets are found in sources such as the regularly updated Transcription Factor Database of the National Center for Biotechnology Information at the National Library for Medicine and Faisst and Meyer (1991) Nucleic Acids Research 20, 3-26. Preferred pairs are listed in Table 1 below.

Table 1

Factor[1]	Binding Sequence[3]
AAF	TTTCATATTACTCT SEQUENCE ID NO:1
AhR	TGCGTGAGAAGA SEQUENCE ID NO:2
Ap1	TGASCTMAA
AP2	CCCMNSSS
AP3	TGTGGWWW
AP4	YCAGCTGYGG SEQUENCE ID NO:3
AR	AGAACANNNTGTTCT SEQUENCE ID NO:4
ARP-1	TGANCCCTTGACCCCT SEQUENCE ID NO:5
ATF	TGACGYMR
BGP1	GGGGGGGGGGGGGGG SEQUENCE ID NO:6
BSAP	GACGCANYGRWNNNMG SEQUENCE ID NO:7
CBF	ACACCCAAATATGGCGAC SEQUENCE ID NO:8
C/EBP	GTGGWWWG
CF1	ANATGG
COUP	GTGTCAAAGGTCA SEQUENCE ID NO:9
CP1	YNNNNNNRRCCAATCANYK SEQUENCE ID NO:10
CP2	YAGYNNNRRCCAATCNNNR SEQUENCE ID NO:11
CTCF	CCCTC

DBP	TGATTTTGT	
E2A	RCAGNTG	
E2B	TGCAAYA	
E2F	TTTTSSCGS	
E4F	TGACGTAAC	
EGR-1	CGCCCSCGC	
EGR-2	CCGCCCCCGC	SEQUENCE ID NO:12
ER	AGGTCANNNTGACCT	SEQUENCE ID NO:13
v-ErbA	GTGTCAAAGGTCA	SEQUENCE ID NO:14
ETF	CAGCCCCCGCGCAGC	SEQUENCE ID NO:15
Ets-1	SMGGAWGY	
F-ACT1	TGGCGA	
GATA-1	WGATAR	
GATA-2	WGATAR	
GATA-3	WGATAR	
GCF	SCGSSSC	
GHF-1	WTATYCAT	
GHF-5	WTATYCAT	
GHF-7	WTATYCAT	
GR	AGAACANNNTGTTCT	SEQUENCE ID NO:16
H1TF2	GCACCAATCACAGCGCGC	SEQUENCE ID NO:17
H2RIIBP	TCAGGTCACAGTGACCTGA	SEQUENCE ID NO:18
H2TF1	TGGGGATTCCCCA	SEQUENCE ID NO:19
H-APF-1	CTGGRAA	
HNF-1	GTTAATNATTAAC	SEQUENCE ID NO:20
vHNF-1	GTTAATNATTAAC	SEQUENCE ID NO:20
HNF-3A	TATTGAYTTWG	SEQUENCE ID NO:21
HNF-3B	TATTGAYTTWG	SEQUENCE ID NO:21
HNF-3C	TATTGAYTTWG	SEQUENCE ID NO:21

HNF-4	KGCWARGKYCAY	SEQUENCE ID NO:22
HSF	NGAANNGAANNGAAN	SEQUENCE ID NO:23
IAF	GCCATCTGCT	SEQUENCE ID NO:24
IREBF-1	CGGGAAATGGAAACTG	SEQUENCE ID NO:25
IRBP	AGTGCACT	
ISGF1	CTTTCAGTTT	SEQUENCE ID NO:26
ISGF2	CTTTCTCTTT	SEQUENCE ID NO:27
ISGF3	GCTTCAGTTT	SEQUENCE ID NO:28
KBF-1	TGGGGATTCCCCA	SEQUENCE ID NO:29
Ker1	GCCTGCAGGC	SEQUENCE ID NO:30
LFB3	GTTAATNATTAAC	SEQUENCE ID NO:31
LIT-1	GCGCCCTTTGGACCT	SEQUENCE ID NO:32
LyF-1	PPTGGGAGR	
MBF-1	YTAAAAATAAYYY	SEQUENCE ID NO:33
MBF-I	TGCRCRC	
MBP-1	TGGGGATTCCCCA	SEQUENCE ID NO:34
MCBF	CATTCCT	
MEF-2	YTAWAAATAR	SEQUENCE ID NO:35
MEP-1	TGCRCNC	
MR	AGAACANNNTGTTCT	SEQUENCE ID NO:36
Myb	YAACKG	
Myc	CACGTG TCTCTTA	
MyoD	CAACTGAC	
NF1	YGGMN$_x$GCCAA where x = 5 or 6 N nucleotides	SEQUENCE ID NO:37
NF-AT	GGAGGAAAAACTGTTTCAT	SEQUENCE ID NO:38
NF-E2	TGACTCAG	
NF-D	GATGGCGG	
NF-GMa	GRGRTTKCAY	SEQUENCE ID NO:39
NF-GMb	TCAGRTA	

NF-IL6	TKNNGNAAK
NFxB	GGGAMTNYCC SEQUENCE ID NO:40
NF-W1	GTTGCATC
NF-W2	GTTGCATC
NGF1-B	AGGTCATGACCT SEQUENCE ID NO:41
Oct-1	ATGCAAAT
Oct-2	ATGCAAAT
Oct-4	ATGCWAAT
Oct-6	ATGCAAAT
Pax-1	CACCGTTCCGCTCTAGATATCTC SEQUENCE ID NO:42
PCF	AGAAAGGGAAAGGA SEQUENCE ID NO:43
PEA3	AGGAAR
PPAR	AGGTCA
PR	AGAACANNNTGTTCT SEQUENCE ID NO:44
PRDI-BF1	AAGTGAAAGT SEQUENCE ID NO:45
PTF1	ATGGGAN$_x$CTCAGCTGTGC where x = 1 or more N nucleotides SEQUENCE ID NO:46
Pu.I	AGAGGAACT
PuF	GGGTGGG
RAR	AGGTCATGACCT SEQUENCE ID NO:47
RFX	CCCCTAGCAACAGATG SEQUENCE ID NO:48
RVF	AAGATAAAACC SEQUENCE ID NO:49
SIF	CCCGTM
Sp1	KRGGCTRRK
SRF	GGATGTCCATATTAGGACATCT SEQUENCE ID NO:50
TBP	TATAAA
TCF-1	MAMAG
TCF-2α	SAGGAAGY
TEF-1	AAGYATGCA

TEF-2	GGGTGTGG
TGT3	AAGTGTTTGC SEQUENCE ID NO:51
TIN-1	AGGAAGTTCC SEQUENCE ID NO:52
WT-ZFP	CGCCCCCGC
XF1/2	TCTTCTCACGCAACT SEQUENCE ID NO:53
XPF-1	CACCTGNNNNTTTCCC SEQUENCE ID NO:54
YB-1	ATTTTTCTGATTGGCCAAAG SEQUENCE ID NO:55
Epstein-Barr Virus EBNA (B958 strain)	GGT TAG CAT ATG CTA ACC A SEQUENCE ID NO:56
Epstein-Barr Virus BZLF (B958 strain)	T TAG CAA TG
Human CBF-1	CGTGGGAA (EpsteinBarr Virus cis-element)
Human Papilloma Virus E2 (strain 6)	A CCG AAA ACG GTG T SEQUENCE ID NO:57
Herpes Simplex Virus Type 1 VP16	ATG CTA ATG ATA SEQUENCE ID NO:58
HIV TAT	GGG TCT CTC TGG TTA GAC CAG ATC TGA GCC TGG GAG CTC TCT GGC TAA CTA GGG AAC CCA (TAR RNA SEQUENCE) SEQUENCE ID NO:59

The disclosed methods and kits involve reconstituting, in vitro, sequence-specific transcription factor-nucleic acid interactions, and challenging the reconstitution with candidate therapeutics. Preferred applications of the method include gene transcriptional regulation where at least one transcription factor and corresponding gene or gene regulatory region have been molecularly cloned. The methods involve forming a mixture of a labelled protein comprising at least a portion of a transcription factor, a nucleic acid conjugated to a ligand, a candidate pharmacological agent and a receptor immobilized on a solid substrate.

The labelled protein comprises at least a portion of a transcription factor and a label, the portion being sufficient to permit sequence-specific binding, direct or indirect, of the labelled protein to the nucleic acid conjugate. The portion is

usually at least about 20, more usually at least about 40, most usually at least about 80 amino acids in length and includes residues sufficient to provide the protein with sequence-specificity similar to that of the native transcription factor. Frequently, the labelled protein will include the entire transcription factor. The labelled protein is typically capable of binding the nucleic acid conjugate with an equilibrium constant at least about 10^4 M^{-1}, preferably at least about 10^6 M^{-1}, more preferably at least about 10^8 M^{-1} and not less than six, preferably not less than four, more preferably not less than two orders of magnitude less than the binding equilibrium constant of the native transcription factor under similar conditions.

Preferred transcription factor portions capable of imparting the requisite binding specificity and affinity are readily identified by those skilled in the art. A wide variety of molecular and biochemical methods are available for generating preferred portions, see e.g. Molecular Cloning, A Laboratory Manual (2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor), Current Protocols in Molecular Biology (Eds. Aufubel, Brent, Kingston, More, Feidman, Smith and Stuhl, Greene Publ. Assoc., Wiley-Interscience, NY, NY, 1992) or that are otherwise known in the art. For example, deletion mutants are screened for sequence-specific binding directly using a label or through gel shift analysis.

The labelled protein also comprises a label which is used to detect labelled protein-nucleic acid complexes. A wide variety of labels may be employed - essentially any label that provides for detection of the labelled protein when complexed to the nucleic acid conjugate. The label may provide for direct detection as radioactivity, luminescence, optical or electron density, etc. or indirect detection such as an epitope tag, an enzyme, etc. The label may be appended to the protein e.g. a phosphate group comprising a radioactive isotope of phosphorous, or incorporated into the protein structure, e.g. a methionine residue comprising a radioactive isotope of sulfur.

The protein may also comprise additional components depending upon the assay reagents and conditions. For example, it may be desirable that the protein be a fusion product of the transcription factor portion and another polypeptide, e.g. a polypeptide that is capable of providing or enhancing sequence-specific nucleic acid binding or stability under assay conditions.

The nucleic acid conjugate comprises a nucleic acid coupled to a ligand. The nucleic acid is usually linear and double-stranded DNA or RNA, particularly in the case of retroviral transcription factor binding sites, though circular plasmids or other nucleic acids or structural analogs may be substituted so long as transcription factor sequence-specific binding is retained. In some applications, supercoiled DNA provides optimal sequence-specific binding and is preferred. The nucleic acid may be of any length amenable to the assay conditions and requirements. Typically the nucleic acid is between 8 bp and 5 kb, preferably between about 12 bp and 1 kb, more preferably between about 18 bp and 250 bp, most preferably between about 27 and 50 bp.

The nucleic acid has a sequence at least a portion of which is common to the gene or gene regulatory region to which the native transcription factor normally binds. The portion may be continuous or segmented and shares sufficient sequence and sequence similarity with the gene or gene regulatory region to provide sequence-specific binding of the labelled protein. Typically, this binding site portion of the nucleic acid constitutes at least about 4, preferably at least about 6, more preferably at least about 8 nucleotides. Additional nucleotides may used to provide structure which enhances or decreased binding or stability, etc. For example, combinatorial DNA binding can be effected by including two or more DNA binding sites for different or the same transcription factor on the oligonucleotide. This allows for the study of cooperative or synergistic DNA binding of two or more factors, e.g. HPV E1 and E2 bind cooperatively to DNA by virtue of their protein-protein interaction. In addition, the nucleic acid can comprise a cassette into which transcription factor binding sites are conveniently spliced for use in the subject assays. An exemplary cassette showing how new DNA binding sites can be exchanged in a simple fashion within a common 30-mer sequence appears in Table 2 below.

Table 2.

EBV EBNA-1 site SEQUENCE ID NO:60	GGA TCT GGT TAG CAT ATG CTA ACC AGG ATC
HPV E2 substituted SEQUENCE ID NO:61	GGA TCT GGT ACC GAA AAC GGT ACC AGG ATC

EBV BZLF-1 substituted SEQUENCE ID NO:62	GGA TCT GGT TAG TTA GCA ATG ACC AGG ATC
Nf-kB and homologs SEQUENCE ID NO:63	GGA TCT GGT TAG GGG ATT TCC ACC AGG ATC
HSV VP16 cis-element SEQUENCE ID NO:64	GGA TCT GGT TAT GCT AAT GAT ATC AGG ATC

The ligand of the nucleic acid conjugate is capable of specifically binding the immobilized receptor. The ligand-receptor binding is specific enough to provide a maximized and at least measurable signal to noise ratio (receptor mediated vs. non-specific retention of the label on the substrate). The nucleic acid conjugate is typically capable of binding the receptor with an affinity of at least about 10^5 M^{-1}, preferably at least about 10^6 M^{-1}, more preferably at least about 10^8 M^{-1}. In a preferred embodiment, a plurality of ligands are capable of binding each receptor. Exemplary ligand-receptor pairs include biotin and avidin, antigen and antibody, sugar and lectin, ion and chelator, etc.

The receptor is immobilized on a solid substrate which may be any solid from which the unbound labelled protein may be conveniently separated. The solid substrate may be made of a wide variety of materials and in a wide variety of shapes, e.g. microtiter plate, microbead, dipstick, resin particle, etc. The substrate is chosen to maximize signal to noise ratios, primarily to minimize background binding, for ease of washing and cost. For example, beads with iron cores may be readily isolated (washed) using magnets.

The mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds; preferably small organic compounds. Small organic compounds have a molecular weight of more than 50 yet less than about 2,500, preferably less than about 1000, more preferably, less than about 500. Candidate agents comprise functional chemical groups necessary for structural interactions with proteins and/or DNA, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of said functional chemical groups, more preferably at least three. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with

one or more of the forementioned functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof, and the like.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means. In addition, known pharmacological agents may be subject to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc., to produce structural analogs.

In addition to the labelled protein, nucleic acid conjugate, candidate agent and immobilized receptor, the mixture usually includes additional reagents, such as salts, buffers, etc. to facilitate optimal receptor-ligand and protein-nucleic acid binding. Auxiliary proteins or portions thereof may also be included to mediate, facilitate or otherwise enhance sequence-specific protein-nucleic acid binding. For example, sequence-specific binding of a number of viral transcription factors is enhanced when complexed with one or more cellular proteins, e.g. Oct1 and HCF in the case of HSV's VP16. Other exemplary auxiliary proteins include CBF1, for EBNA-2 binding, ATF-2 or AP-1 for Adenovirus E1A binding, etc.

A variety of other reagents may also be included in the mixture. These include reagents like detergents which may be used to reduce non-specific or background protein-substrate, nucleic acid-substrate, protein-protein and protein-DNA interactions, etc. Also, reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, antimicrobial agents, etc. may be used.

The mixture is incubated under conditions whereby the receptor is bound to the ligand and, but for the presence of said candidate pharmacological agent, the labelled protein is sequence-specifically bound to the nucleic acid. The mixture

components can be added in any order that provides for the requisite bindings. For example, the nucleic acid conjugate may be added first and prebound to the substrate through ligand-receptor binding before the labelled protein is added. Alternatively, the nucleic acid conjugate and labelled protein can be preincubated and complexed and then added to the substrate for attachment, or the various mixture components and reagents can be added to the substrate simultaneously. Adding the protein and nucleic acid components together may be thermodynamically advantageous in that in some nucleic acid-protein complexes, initial binding may be favored by a soluble, unrestrained nucleic acid molecule.

Incubations may be performed at any temperature which facilitates optimal binding, typically between 4 and 40°C, more commonly between 15 and 40°C. Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening. Typically, protein-nucleic acid and receptor-ligand pairs are coincubated between .1 and 10 hours, preferably less than 5 hours, more preferably less than 2 hours each; of course, the incubations may and preferably do run simultaneously.

After receptor-ligand and protein-nucleic acid binding have occurred, a fraction comprising labelled protein which is not sequence-specifically bound is separated from the solid substrate. This step may be accomplished in a variety of ways including removing a bead or dipstick from a reservoir, emptying or diluting reservoir such as a microtiter plate well, rinsing a bead, particle, chromatographic column or filter with a wash solution or solvent. Typically, the separation step will include an extended rinse or wash or a plurality of rinses or washes. For example, where the solid substrate is a microtiter plate, the wells may be washed several times with a washing solution, which typically includes those components of the incubation mixture that do not participate in specific binding such as salts, buffer, detergent, nonspecific protein, etc.

After separating the unbound fraction from the solid substrate, the presence of bound nucleic acid-protein complex is detected via the labeled protein. A variety of methods may be used to detect the label depending on the nature of the label and other assay components. For example, the label may be detected bound to the solid substrate or a portion of the bound complex containing the label may be separated from the solid substrate, and thereafter the label detected. Labels

may be directly detected through optical or electron density, radiative emissions, nonradiative energy transfers, etc. or indirectly detected with antibody conjugates, etc. For example, in the case of radioactive labels, emissions may be detected directly, e.g. with particle counters or indirectly, e.g. with scintillation cocktails and counters.

Candidate agents shown to modulate transcription complex formation provide valuable reagents to the pharmaceutical and agricultural industries for cellular, plant, field crop, animal and human trials.

The methods are particularly suited to automated high throughput drug screening. In a preferred embodiment, the individual sample incubation volumes are less than about 500 ul, preferably less than about 250 ul, more preferably less than about 100 ul. Such small sample volumes minimize the use of often scarce candidate agent, expensive transcription complex components, and hazardous radioactive waste. Furthermore, the methods provide for automation, especially computerized automation. Accordingly, the method steps are preferably performed by a computer-controlled electromechanical robot. While individual steps may be separately automated, a preferred embodiment provides a single computer-controlled multifunction robot with a single arm axially rotating to and from a plurality of work stations performing the mixture forming, incubating and separating steps. The computer is loaded with software which provides the instructions which direct the arm and work station operations and provides input (e.g. keyboard and/or mouse) and display (e.g. monitor) means for operator interfacing.

In a particular embodiment, the arm retrieves and transfers a microtiter plate to a liquid dispensing station where measured aliquots of each an incubation buffer and a solution comprising one or more candidate agents are deposited into each designated well. The arm then retrieves and transfers to and deposits in designated wells a measured aliquot of a solution comprising a labeled transcription factor protein. After a first incubation period, the liquid dispensing station deposits in each designated well a measured aliquot of a biotinylated nucleic acid solution. The first and/or following second incubation may optionally occur after the arm transfers the plate to a shaker station. After a second incubation period, the arm transfers the microtiter plate to a wash station where the unbound contents of each

well is aspirated and then the well repeatedly filled with a wash buffer and aspirated. Where the bound label is radioactive phosphorous, the arm retrieves and transfers the plate to the liquid dispensing station where a measured aliquot of a scintillation cocktail is deposited in each designated well. Thereafter, the amount of label retained in each designated well is quantified.

In more preferred embodiments, the liquid dispensing station and arm are capable of depositing aliquots in at least eight wells simultaneously and the wash station is capable of filling and aspirating ninety-six wells simultaneously. Preferred robots are capable of processing at least 640 and preferably at least about 1,280 candidate agents every 24 hours, e.g. in microtiter plates.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

GENERIC PROTOCOL FOR TRANSCRIPTION FACTOR-DNA BINDING ASSAY

1. Reagents:

- Neutralite Avidin: 20 μg/ml in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hr, RT.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 0.25 mM EDTA, 1% glycerol, 0.5 % NP-40, 50 mM BME, 1 mg/ml BSA, cocktail of protease inhibitors.
- ^{33}P Full-Length Transcription Factor 10x stock: 1-5x10^{-8} "cold" protein comprising unlabeled protein comprising transcription factor portion supplemented with 100,000-500,000 cpm of labeled protein (Beckman counter). Placed in the 4°C microfridge during screening.
- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2 mM $NaVo_3$ (Sigma # S-6508) in 10 ml of PBS.
- Oligonucleotide stock: (specific biotinylated). Biotinylated oligo at 1-100 pmole/μl, including transcription factor binding site:
(BIOTIN)-oligo : e.g. derived from Table 1.

anti-sense: derived as reverse complement of target oligo above.

2. Preparation of assay plates:

- Coat with 100 μl of stock N-Avidin per well overnight at 4°C.
- Wash 2X with 200 μl PBS.
- Block with 150 μl of blocking buffer.
- Wash 2X with 200 μl PBS.

3. Assay:

- Add 40 μl assay buffer/well.
- Add 10 μl compound or extract.
- Add 10 μl ^{33}P-labelled protein (10,000-50,000 cpm/well; 10^{-10}-10^{-8} M final concentration).
- Shake at RT for 15 min.
- Incubate additional 45 min. at 25°C.
- Add 40 μl oligo mixture (1.0 pmoles/40 ul in assay buffer)
- Incubate 1 hr at 25°C.
- Stop the reaction by washing 4X with 200 μl PBS.
- Add 150 μl scintillation cocktail.
- Count in Topcount.

PROTOCOL FOR EPSTEIN BARR VIRUS EBNA-1 BINDING ASSAY

1. Reagents:

- Neutralite Avidin: 20 μg/ml in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hr, RT.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 0.25 mM EDTA, 1% glycerol, 0.5 % NP-40, 50 mM BME, 1 mg/ml BSA, cocktail of protease inhibitors.
- ^{33}P EBNA 10x stock: $3x10^{-8}$ "cold" EBNA (M.W. ~40,000 for dimer) supplemented with 200,000-250,000 cpm of labeled EBNA-1 (Beckman counter). This is to be placed in the 4°C microfridge during screening.
- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2 mM $NaVo_3$ (Sigma # S-6508) in 10 ml of PBS.

- Oligonucleotide stock: (specific biotinylated). Biotinylated oligo at 17 pmole/μl, EBNA site TO889/832:
(BIOTIN)-GGA TCT GGT TAG CAT ATG CTA ACC AGG ATC
(SEQUENCE ID NO:60); anti-sense-GAT CTT GGT TAG CAT ATG CTA ACC AGA TCC (SEQUENCE ID NO:65)

2. Preparation of assay plates:

- Coat with 100 μl of stock N-Avidin per well overnight at 4°C.
- Wash 2X with 200 μl PBS.
- Block with 150 μl of blocking buffer.
- Wash 2X with 200 μl PBS.

3. Assay:

- Add 40 μl assay buffer/well.
- Add 10 μl compound or extract.
- Add 10 μl ^{33}P-EBNA-1 (20,000-25,000 cpm/0.3 pmoles/well = $3x10^{-9}$ M final concentration).
- Shake at RT for 15 min.
- Incubate additional 45 min. at RT.
- Add 40 μl oligo mixture (1.0 pmoles/40 ul in assay buffer)
- Incubate 1 hr at RT.
- Stop the reaction by washing 4X with 200 μl PBS.
- Add 150 μl scintillation cocktail.
- Count in Topcount.

PROTOCOL FOR EPSTEIN BARR VIRUS BZLF-1 BINDING ASSAY

1. Reagents:

- Neutralite Avidin: 20 μg/ml in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hr, RT.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 0.25 mM EDTA, 1% glycerol, 0.5 % NP-40, 50 mM BME, 1 mg/ml BSA, cocktail of protease inhibitors.
- ^{33}P Full-Length BZLF 10x stock: $1x10^{-8}$ "cold" BZLF supplemented with 180,000-220,000 cpm of labeled BZLF (Beckman counter), resulting in an approx. specific activity of 180,000-220,000 cpm /1 pmole (M.W. ~54,000 for dimer).

The protein stock solution contains 70% Et-OH, 30% assay buffer without BSA, and 50 mM BME (final concentration). The protein is to be placed in the 4°C microfridge during screening.

- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2 mM $NaVo_3$ (Sigma # S-6508) in 10 ml of PBS.

- Oligonucleotide stock: (specific biotinylated). Biotinylated oligo at 22 pmole/μl, BZLF site TO855/854:

sense-(BIOTIN) TTAT CTA CAT TAG CAA TGC CTT AGC AAT GTG CAT A (SEQUENCE ID NO:66); anti-sense-TAT GCA CAT TGC TAA GGC ATT GCT AAT GTA GAT A (SEQUENCE ID NO:67)

2. Preparation of assay plates:

- Coat with 100 μl of stock N-Avidin per well overnight at 4°C.
- Wash 2X with 200 μl PBS.
- Block with 150 μl of blocking buffer.
- Wash 2X with 200 μl PBS.

3. Assay:

- Add 40 μl assay buffer/well.
- Add 10 μl compound or extract.
- Add 10 μl ^{33}P-BZLF (18,000-22,000 cpm/0.1 pmoles/well = $1x10^{-9}$ M final concentration).
- Shake at RT for 15 min.
- Incubate additional 45 min. at RT.
- Add 40 μl oligo mixture (1.0 pmoles/40 ul in assay buffer)
- Incubate 1 hr at RT.
- Stop the reaction by washing 4X with 200 μl PBS.
- Add 150 μl scintillation cocktail.
- Count in Topcount.

PROTOCOL FOR HUMAN PAPILLOMA VIRUS 6 E2 BINDING ASSAY

1. Reagents:

- Neutralite Avidin: 20 μg/ml in PBS.

- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hr, 25°C.

- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 0.25 mM EDTA, 1% glycerol, 0.5% NP-40, 50 mM BME, 1 mg/ml BSA, cocktail of protease inhibitors.

- ^{33}P Full-Length E2 10x stock: $1x10^{-8}$ "cold" E2 supplemented with 200,000-300,000 cpm of labeled E2, resulting in an approx. specific activity of 200,000-300,000 cpm /1 pmole (M.W. ~100 kD for dimer). Place in microfridge set at 4°C.

- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2 mM $NaVo_3$ (Sigma # S-6508) in 10 ml of PBS.

- Oligonucleotide stock: (specific biotinylated and sheared salmon sperm (sss)-DNA). Biotinylated oligo at 25 pmole/ml, HPV-E2 1 site TO922/923: (BIOTIN) -CCA GAG TGA CCG AAA ACG GTG TGA GAC C (SEQUENCE ID NO:68); anti-sense- GGT CTC ACA CCG TTT TCG GTC ACT CTG G (SEQUENCE ID NO:69) and sss-DNA at 25 μg/ml in assay buffer.

2. Preparation of assay plates:

- Coat with 100 μl of stock N-Avidin per well overnight at 4°C.
- Wash 2X with 200 μl PBS.
- Block with 150 μl of blocking buffer.
- Wash 2X with 200 μl PBS.

3. Assay:

- Add 40 μl assay buffer/well.
- Add 10 μl compound or extract.
- Add 10 μl ^{33}P-E2 (20,000-30,000 cpm/0.1 pmoles/well = $1x10^{-9}$ M final concentration).
- Shake at 25°C for 15 min.
- Incubate additional 45 min. at 25°C.
- Add 40 μl oligo mixture (1 pmole of biotinylated specific oligo and 1 μg of sss-DNA)
- Incubate 1 hr at 25°C.
- Stop the reaction by washing 4X with 200 μl PBS.

- Add 150 μl scintillation cocktail.
- Count in Topcount.

4. Controls:

a. Non-specific binding (no oligo added)

b. Specific soluble oligo at 80% inhibition

PROTOCOL FOR Nf-kB BINDING ASSAY (p65/p50)

1. Reagents:

- Neutralite Avidin: 50 μg/ml in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hr, RT.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.9, 0.5 mM EDTA, 1% glycerol, 0.5% NP-40, 1 mg/ml BSA, 50 mM BME, cocktail of protease inhibitors.
- ^{33}Pp65/p65/p50 10x stock: $1x10^{-8}$ "cold" p65/p50 ($5x10^{-9}$ M p65 + $5x10^{-9}$ M p50 supplemented with 200,000-300,000 cpm of labeled p65, resulting in an approx. specific activity of 200,000-300,000 cpm /1 pmole. Heterodimer formation is promoted by incubating the mixture for 1 hr at 37°C prior to use.
- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2 mM $NaVo_3$ (Sigma # S-6508) in 10 ml of PBS.
- Biotinylated oligo: 40x stock at 1 pmoles/1 μl in assay buffer.

ELAM 2 site END-126/127: (BIOTIN) -CAA CAG ATT GGG GAT TTC CTC GGT TCC ATT GGG GAT TTC CTC CAG C (SEQUENCE ID NO:70); anti-sense-GC TGA GAG GAA ATC CCC AAT GGA ACC GAG GAA ATC CCC AAT CTG TTG (SEQUENCE ID NO:71)

2. Preparation of assay plates:

- Coat with 100 μl of stock N-Avidin per well overnight at 4°C.
- Wash 2X with 200 μl PBS.
- Block with 150 μl of blocking buffer.
- Wash 2X with 200 μl PBS.

3. Assay:

- Add 40 μl assay buffer/well.

- Add 10 μl compound or extract.

- Add 10 μl ^{33}P-p65/p50 (20,000-30,000 cpm/0.1 pmoles/well = $1x10^{-9}$ M final concentration).

- Shake at RT for 15 min.

- Incubate additional 45 min. at RT.

- Add 40 μl biotinylated oligo (1.0 pmole/40 μl/well) in assay buffer.

- Incubate 1 hr at RT.

- Stop the reaction by washing 4X with 200 μl PBS.

- Add 150 μl scintillation cocktail.

- Count in Topcount.

PROTOCOL FOR Nf-kB BINDING ASSAY (p65/p65)

1. Reagents:

- Neutralite Avidin: 20 μg/ml in PBS.

- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hr, RT.

- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.9, 0.5 mM EDTA, 1% glycerol, 0.5% NP-40, 1 mg/ml BSA, 50 mM BME, cocktail of protease inhibitors.

- ^{33}Pp65/p65 10x stock: $1x10^{-8}$ "cold" p65 supplemented with 200,000-300,000 cpm of labeled p65, resulting in an approx. specific activity of 200,000-300,000 cpm /1 pmole.

- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2 mM $NaVo_3$ (Sigma # S-6508) in 10 ml of PBS.

- Biotinylated oligo: 40x stock at 1 pmoles/1 μl in assay buffer.

ELAM 2 site END-126/127: (BIOTIN) -CAA CAG ATT GGG GAT TTC CTC GGT TCC ATT GGG GAT TTC CTC CAG C (SEQUENCE ID NO:70); anti-sense-GC TGA GAG GAA ATC CCC AAT GGA ACC GAG GAA ATC CCC AAT CTG TTG (SEQUENCE ID NO:72)

2. Preparation of assay plates:

- Coat with 100 μl of stock N-Avidin per well overnight at 4°C.

- Wash 2X with 200 μl PBS.

- Block with 150 μl of blocking buffer.
- Wash 2X with 200 μl PBS.

3. Assay:

- Add 40 μl assay buffer/well.
- Add 10 μl compound or extract.
- Add 10 μl ^{33}P-p65 (20,000-30,000 cpm/0.1 pmoles/well=1x10^{-9} M final concentration).
- Shake at RT for 15 min.
- Incubate additional 45 min. at RT.
- Add 40 μl biotinylated oligo (1.0 pmole/50 μl/well) in assay buffer.
- Incubate 1 hr at RT.
- Stop the reaction by washing 4X with 200 μl PBS.
- Add 150 μl scintillation cocktail.
- Count in Topcount.

PROTOCOL FOR HERPES SIMPLEX VIRUS VP-16 BINDING ASSAY

1. Reagents:

- Neutralite Avidin: 20 μg/ml in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hr, 25°C.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 0.25 mM EDTA, 1% glycerol, 0.5% NP-40, 50 mM BME, 1 mg/ml BSA, cocktail of protease inhibitors.
- ^{33}P Truncated VP-16/HCF/OCT-1 10x stock mix: 1x10^{-8} "cold" VP-16 supplemented with 250,000-300,000 cpm of labeled VP-16, resulting in an approx. specific activity of 250,000-300,000 cpm /1 pmole (M.W. ~18 kD), 50 μl HCF, and 500 ng of OCT-1 per ml of the stock mix. Place in microfridge set at 4°C.
- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2 mM $NaVo_3$ (Sigma # S-6508) in 10 ml of PBS.
- Oligonucleotide stock: (specific biotinylated and sss-DNA). Biotinylated oligo at 25 pmole/ml, HSV-VP-16 TO876/877: sense-Biotin T - GAT AGT CAG GAC TGA ATG CCG TGC ATG CTA ATG ATA TTC TTT GCT TGA TC

(SEQUENCE ID NO:73); anti-sense-GAT CAA GCA AAG AAT ATC ATT AGC ATG CAC GGC ATT CAG TCC TGA CTA TC (SEQUENCE ID NO:74) and sss-DNA at 2.5 μg/ml in assay buffer.

2. Preparation of assay plates:

- Coat with 120 μl of stock N-Avidin per well overnight at 4°C.
- Wash 2X with 200 μl PBS.
- Block with 150 μl of blocking buffer.
- Wash 2X with 200 μl PBS.

3. Assay:

- Add 40 μl assay buffer/well.
- Add 10 μl compound or extract.
- Add 10 μl ^{33}P-VP-16, HCF, OCT-1 stock (25,000-30,000 cpm/ 0.1 pmoles/well = $1x10^{-9}$ M final concentration, 0.5 μl HCF,and 5 ng OCT-1)
- Shake at 25°C for 15 min.
- Incubate additional 45 min. at 25°C.
- Add 40 μl oligo mixture (1 pmole of biotinylated specific oligo and 100 ng of ss-DNA)
- Incubate 1 hr at 25°C.
- Stop the reaction by washing 4X with 200 μl PBS.
- Add 150 μl scintillation cocktail.
- Count in Topcount.

4. Controls for all assays (located on each plate):

a. Non-specific binding (no oligo added)

b. Specific soluble oligo at 80% inhibition.

PROTOCOL FOR HIV TAT BINDING ASSAY

1. Reagents:

- Neutralite Avidin: 20 μg/ml in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hr, RT.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.9, 0.5 mM EDTA, 1% glycerol, 0.5% NP-40, 1 mg/ml BSA, 50 mM BME, cocktail of protease inhibitors.

- ^{33}PTAT10x stock: $1x10^{-8}$ "cold" p65 supplemented with 200,000-300,000 cpm of labeled TAT, resulting in an approx. specific activity of 200,000-300,000 cpm /1 pmole.

- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2 mM $NaVo_3$ (Sigma # S-6508) in 10 ml of PBS.

- Biotinylated oligo: 40x stock at 1 pmoles/1 μl in assay buffer.

TAR RNA site: (BIOTIN) -GGG TCT CTC TGG TTA GAC CAG ATC TGA GCC TGG GAG CTC TCT GGC TAA CTA GGG AAC CCA (SEQUENCE ID NO:59)

2. Preparation of assay plates:

- Coat with 100 μl of stock N-Avidin per well overnight at 4°C.
- Wash 2X with 200 μl PBS.
- Block with 150 μl of blocking buffer.
- Wash 2X with 200 μl PBS.

3. Assay:

- Add 40 μl assay buffer/well.
- Add 10 μl compound or extract.
- Add 10 μl ^{33}P-pTAT (20,000-30,000 cpm/0.1 pmoles/well = 1x10-9 M final concentration).
- Shake at RT for 15 min.
- Incubate additional 45 min. at RT.
- Add 40 μl biotinylated oligo (1.0 pmole/50 μl/well) in assay buffer.
- Incubate 1 hr at RT.
- Stop the reaction by washing 4X with 200 μl PBS.
- Add 150 μl scintillation cocktail.
- Count in Topcount.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of

illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: TULARIK, INC.

(ii) TITLE OF INVENTION: TRANSCRIPTION FACTOR-DNA BINDING ASSAY

(iii) NUMBER OF SEQUENCES: 74

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(E) COUNTRY: USA
(F) ZIP: 94111-4187

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
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(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/235,503
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(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTTCATATTA CTCT 14

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGCGTGAGAA GA 12

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

YCAGCTGYGG 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGAACANNNT GTTCT 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGANCCCTTG ACCCCT 16

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGGGGGGGGG GGGGGG 16

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GACGCANYGR WNNNMG 16

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ACACCCAAAT ATGGCGAC 18

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTGTCAAAGG TCA 13

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

YNNNNNNRRC CAATCANYK 19

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

YAGYNNNRRC CAATCNNNR 19

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCGCCCCCGC 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGGTCANNNT GACCT 15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTGTCAAAGG TCA 13

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGCCCCCGC GCAGC 15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AGAACANNNT GTTCT 15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCACCAATCA CAGCGCGC 18

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TCAGGTCACA GTGACCTGA 19

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGGGGATTCC CCA 13

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTTAATNATT AAC 13

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TATTGAYTTW G 11

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

KGCWARGKYC AY 12

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

NGAANNGAAN NGAAN 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCCATCTGCT 10

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGGGAAATGG AAACTG 16

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTTTCAGTTT 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTTTCTCTTT 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GCTTCAGTTT 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TGGGGATTCC CCA 13

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCCTGCAGGC 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTTAATNATT AAC 13

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GCGCCCTTTG GACCT 15

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

YTAAAAATAA YYY 13

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TGGGGATTCC CCA 13

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

YTAWAAATAR 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

AGAACANNNT GTTCT 15

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /note= "Where this sequence can contain 5 or 6 N nucleotides"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

YGGMNNNNNG CCAA 14

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGAGGAAAAA CTGTTTCAT 19

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GRGRTTKCAY 10

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGGAMTNYCC 10

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

AGGTCATGAC CT 12

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CACCGTTCCG CTCTAGATAT CTC 23

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

AGAAAGGGAA AGGA 14

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AGAACANNNT GTTCT 15

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AAGTGAAAGT 10

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /note= "Where N is one or more nucleotides."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ATGGGANCTC AGCTGTGC 18

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

AGGTCATGAC CT 12

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CCCCTAGCAA CAGATG 16

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

AAGATAAAAC C 11

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GGATGTCCAT ATTAGGACAT CT 22

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

AAGTGTTTGC 10

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

AGGAAGTTCC 10

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TCTTCTCACG CAACT 15

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CACCTGNNNN TTTCCC 16

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

ATTTTTCTGA TTGGCCAAAG 20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GGTTAGCATA TGCTAACCA 19

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ACCGAAAACG GTGT 14

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

ATGCTAATGA TA 12

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGGTCTCTCT GGTTAGACCA GATCTGAGCC TGGGAGCTCT CTGGCTAACT AGGGAACCCA 60

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGATCTGGTT AGCATATGCT AACCAGGATC 30

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GGATCTGGTA CCGAAAACGG TACCAGGATC 30

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GGATCTGGTT AGTTAGCAAT GACCAGGATC 30

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GGATCTGGTT AGGGGATTTC CACCAGGATC 30

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGATCTGGTT ATGCTAATGA TATCAGGATC 30

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GATCTTGGTT AGCATATGCT AACCAGATCC 30

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

TTATCTACAT TAGCAATGCC TTAGCAATGT GCATA 35

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

TATGCACATT GCTAAGGCAT TGCTAATGTA GATA 34

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CCAGAGTGAC CGAAAACGGT GTGAGACC 28

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GGTCTCACAC CGTTTTCGGT CACTCTGG 28

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CAACAGATTG GGGATTTCCT CGGTTCCATT GGGGATTTCC TCCAGC 46

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GCTGAGAGGA AATCCCCAAT GGAACCGAGG AAATCCCCAA TCTGTTG 47

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GCTGAGAGGA AATCCCCAAT GGAACCGAGG AAATCCCCAA TCTGTTG 47

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GATAGTCAGG ACTGAATGCC GTGCATGCTA ATGATATTCT TTGCTTGATC 50

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GATCAAGCAA AGAATATCAT TAGCATGCAC GGCATTCAGT CCTGACTATC 50

WHAT IS CLAIMED IS:

1. A method of screening a chemical library for pharmacological agents active at the level of gene transcription, said method comprising the steps of:

forming a mixture by combining a labelled protein comprising a portion of a transcription factor and a label, a nucleic acid conjugate, a candidate pharmacological agent and a receptor immobilized on a solid substrate, wherein said nucleic acid conjugate comprises a nucleotide sequence and a ligand capable of specifically binding said receptor,

incubating said mixture under conditions whereby said receptor is bound to said ligand and, but for the presence of said candidate pharmacological agent, said labelled protein is sequence-specifically bound to said nucleic acid conjugate;

separating from said solid substrate a fraction of said mixture, which fraction comprises said labelled protein if said labelled protein is not sequence-specifically bound to said nucleic acid conjugate;

detecting the presence or absence of said label on said solid substrate;

wherein the absence of said label on said solid substrate indicates that said candidate pharmacological agent is pharmacologically active at the level of gene transcription.

2. A method according to claim 1, wherein said labelled protein is capable of directly sequence-specifically binding said nucleic acid conjugate with a binding affinity of at least 10^6 M^{-1}.

3. A method according to claim 1, wherein said mixture is formed by combining said labelled protein, said nucleic acid conjugate, said candidate pharmacological agent, said receptor immobilized on a solid substrate and an auxiliary protein portion,

4. A method according to claim 1, wherein said transcription factor portion is a viral transcription factor portion.

5. A method according to claim 1, wherein said transcription factor portion is a bacterial transcription factor portion.

6. A method according to claim 1, wherein said transcription factor portion is a mono- or dicotyledonous plant transcription factor portion.

7. A method according to claim 1, wherein said transcription factor portion is a fungal transcription factor portion

8. A method according to claim 1, wherein said receptor is avidin and said ligand is biotin.

9. A method according to claim 1, wherein said forming step and said separating step are performed at least in part by a computer controlled electromechanical robot comprising an axial rotatable arm and said solid substrate is a portion of a well of a microtiter plate.

10. A kit for screening a chemical library for pharmacological agents active at the level of gene transcription, said kit comprising:

a solid substrate,

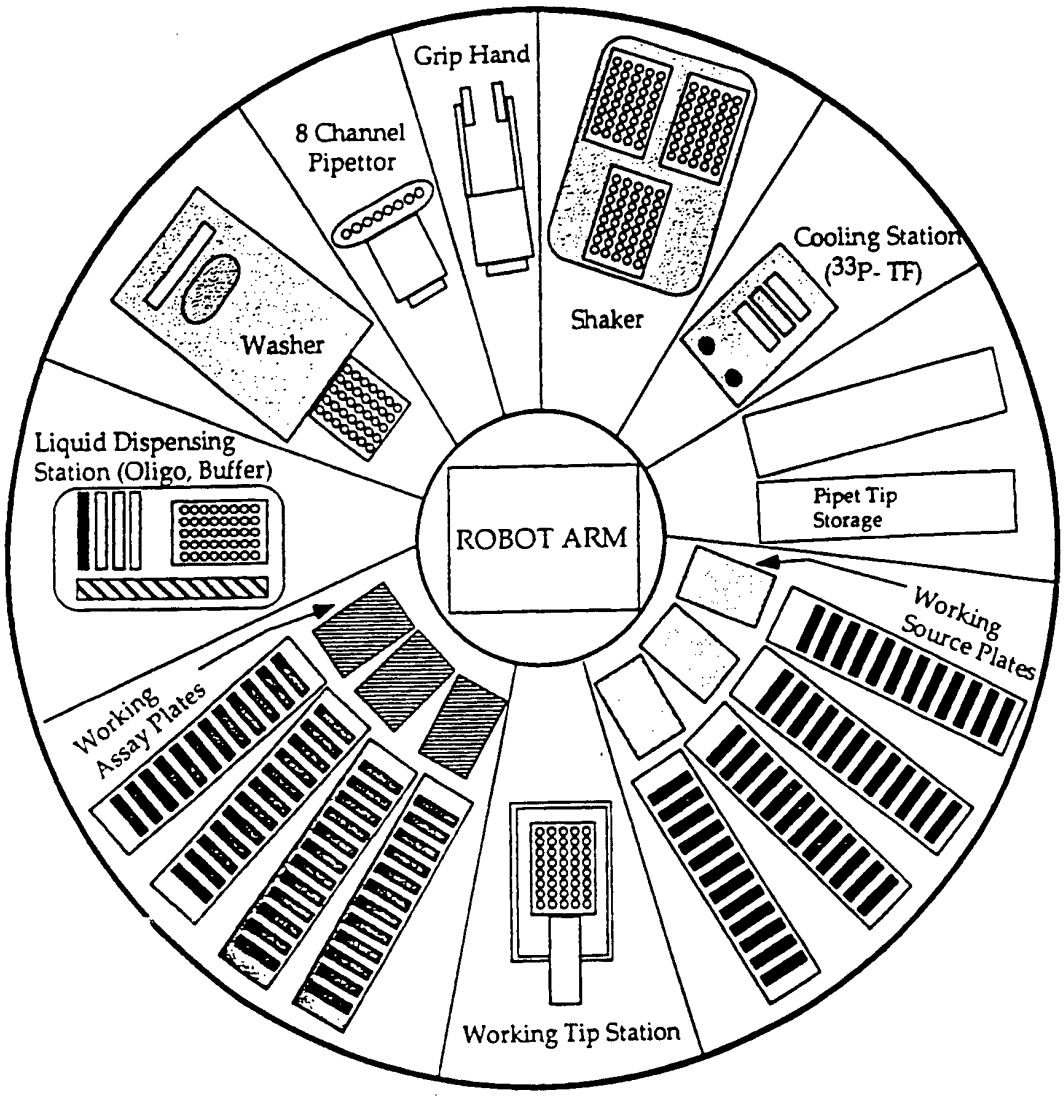
a labelled protein comprising a portion of a transcription factor and a label,

a candidate pharmacological agent

a receptor immobilized on said solid substrate,

a nucleic acid conjugate comprising a nucleotide sequence and a ligand capable of specifically binding said receptor.

FIGURE 1



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/05265

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68
US CL :436/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,200,051 (COZZETTE ET AL) 06 April 1993, see entire document.	1-10
Y	Nucleic Acids Research, Volume 19, Number 10, issued 1991, Jost et al., "Study of Protein-DNA Interactions by Surface Plasmon Resonance (Real Time Kinetics)", page 2788, see page 2788.	1-10
Y	Proceedings of the American Association for Cancer Research, Volume 32, issued March 1991, Gambari et al., "Studies on the Inhibition of Interaction Between Nuclear Factors and Specific DNA Sequences by Antitumor Antibiotics and Related Compounds", page 333, column 2, abstract no. 1978, see entire abstract.	1-10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 JUNE 1995	12 JUL 1995

Name and mailing address of the ISA/US	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/05265

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Trends in Biotechnology, Volume 11, issued January 1993, Peterson et al., "Transcription Factor Based Therapeutics: Drugs of the Future?", pages 11-18, see entire document.	1-10
Y	US, A, 4,816,730 (WILHELM, JR. ET AL) 28 March 1989, see entire document.	1, 9

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG
search terms: transcription factor?, DNA binding protein?, DNA binding factor?, assay?, screen?, avidin, biotin, axial, rotatable, robot?